

Comparison of the efficacy of dietary lactose and administration via drinking water to reduce *Salmonella* colonization in broiler chickens

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Abstract

The ability of dietary lactose and administration via drinking water to reduce *Salmonella* colonization of the crop and cecal contents of young chickens was evaluated. Chicks treatment consisted of dietary lactose treated group and administration lactose in drinking water treated group and control, in each group (150) chicks were placed. Chicks were challenged per os at 3 days of age with 10^6 cfu of *Salmonella*, bird samples were taken at day 7, 14, 21 and their crop and cecal contents were analyzed for *Salmonella* isolation rate from crop, number of *Salmonella* per gram from cecal content, and fecal shedding. The results showed that there is a total significant decrease in the isolation rate of *Salmonella* from crop and significant decrease in the number of *Salmonella* per gram of cecal contents and total significance decrease in the fecal shedding in the treated groups comparing to the control and there is no difference by using dietary lactose or administration via drinking water to reduce *Salmonella* colonization of broiler chicks and there is no divers effect on the body weight between all groups. (no significant difference).

Introduction

The growth of desirable bacteria in the digestive tract of chickens has been promoted as a mechanism for reducing undesirable pathogenic bacteria of the intestine. There are several studies showing that effects of lactose (1,2,3, 4) in the diet for ability to control *Salmonella* in poultry, however, Nisbet et al.(1994) (5), observed little reduction of *Salmonella* colonization 10 day old broilers fed 2% dietary lactose. In spite of chickens lack lactase but chicks consuming lactose had ceca that were distended as compared with the controls and the cecal contents were foamy (1,4). It well documented that chicks are highly susceptible to *Salmonella* spp. colonization than older chickens (6), *Salmonella* colonize through the adhesive filamentous appendages which are called fimbriae and the type is based on the haema-gglutinating properties of the bacteria, type 1 fimbriae agglutinate RBCs of most mammalian species, this agglutination is inhibited by mannose and the agglutinating activity is termed mannose sensitive (7). Type 2 fimbriae are also adhesive appendages but are insensitive to mannose (8). *Salmonella* spp.

Have type 1 and 2 fimbriae but type 1 is the most prevalent. These susceptibility of chicks due to the lack of the mature micro flora there for providing chicks anaerobic bacterial cultures from cecal contents of mature chickens increases resistance to colonization by *Salmonella* (9), which now called probiotics another interested approach is prebiotics that means substrates which give via diet or water to enhance the intestinal flora of chicks such as carbohydrates (mannose, lactose)(10,11,12), third approach is synbiotic which is mean mixture of prebiotics and probiotics giving together to chicks (2,11,13,). Chickens intestinal micro flora well decrease *Salmonella* colonization of chicks intestine by competing for attachment sites on the intestinal wall (14) or by producing bacteriostatic or cidal short chain VFA(1,2), that inhibited the growth of enteropathgens (15). The addition of lactose to the feed or water further enhances *Salmonella* spp. colonization resistance in broilers inoculated with anaerobic culture of cecal micro flora (2, 16). *Salmonella* spp. Found in poultry appears to colonize

the digestive tract during the first few weeks of life and susceptibility to colonization decrease with age of the birds, once these bacteria establishes growth in the digestive tract, it can remain there throughout the chick's life while being shed in the feces (17). Also other types of sugars were tested for prevention of *Salmonella* such as lactose or mannose which reduce the bacterial adherence to the intestinal epithelial cells because for *Salmonella* to occur the bacteria must first colonize in host intestinal epithelium which this process has been recognized as

Materials and Methods

1-Salmonella Source

Salmonella typhimurium isolate were obtained by isolation from broilers, *Salmonella* was growth in tryptic soy broth for 8-12 hr then made a serial dilution and spread plating, preparing *Salmonella* challenge dose it was according to (19).

2-Animal Source

One day commercial broiler chicks were obtained from commercial hatchery

a vital step in the infectious process (18), or enhances the microflora population and its static or cidal substrates but with no such results of glucose, galactose and arabinose (7,10,11,13,17), and if attachment doesn't occur they are expelled by the host physical mechanical defense mechanisms such as peristaltic movement and mucous secretions. The objective of the present study was to examine the role or degree of dietary lactose and administration of lactose in drinking water in providing resistance to *Salmonella* in broilers.

and placed with in isolated rooms in floor pens with new litter each group was (150) chicks, standard diet was formulated to meet the nutrient levels recommended by the national research council (20). birds feeding were provided ad libitum, all three groups are submitted to the same schedule of vaccination against ND and IBD.

Composition of broiler feed

Material	Amount/kg
Corn	563
Soybean	356
Premix	10
Methionine	1.2
Fat	10
Lime	12
Cal .phosphate	5
Salt	3

Diet analysis

Protein %	Meta. Energy/Kcalorie	Ca
22	3060	0.42

Water was provided for ad libitum consumption, all chicks kept under controlled environment.

3-Culture Procedure

For crop and cecal contents examination bird samples were killed by cervical dislocation and for fecal shedding cotton swab were taken from live chicks, crop ,

cecal contents and cotton swabs were immersed in selenite broth and mixed well then serial dilutions were made (for cecal content) then all samples incubated for 18 hr then each selenite broth tube with growth was plated on brilliant green agar for 24 hr at 37c and examined for presence of lactose negative *Salmonella* colonies

and confirmed biochemically and serologically.

4-Experimental Design

At chicks arrival cotton swabs were obtained from samples of chicks to confirm the chicks were negative to *Salmonella* infection, birds were assigned randomly to 3 groups' each group contain 150 chicks. Tap water alone (control) or tap water containing 2.5% lactose and dietary lactose 5% were provided for first 3 days of life then at 8 days of age also for 3 additional days. Birds were killed and

Results and Discussion

Table(1) showed that the isolation rate of *Salmonella* from crop samples in three groups were with no differences between comparable groups at 7,14, 21 days of chicks life this may be due to the presence of *Lactobacilli* in high numbers in the crop(21), which have inhibition effect on the growth of *Salmonella* and the unfavorable acidic condition in this site of digestive tract for *Salmonella* growth (22,23,24), but numerically the control group was higher than two treated groups at 7 and 14 days of age, and comparing the total positive samples of treated groups were significantly differ than control this may important at processing to reduce carcass contamination this result of reducing the positive samples in the treated groups may refer that the lactose were had an desirable effects on the growth of *Lactobacilli* and enhance the *Salmonella* colonization resistance and decreasing PH of the crop as lactose serve as a fermentation substrate in crops(12), while at 21 day of life there is no difference in the isolation rate from the three groups this may refer to the developing of the *lactobacilli* in the crop with aging. Table(2) showed that *Salmonella* number expressed by \log_{10} were at 7 day of age were no differences between comparable three groups may be due to the lack of mature micro flora at the mechanical colonization site of *Salmonella* (the cecum) and raising the chicks under controlled condition and new litter may delay the development of intestinal flora which prevent the colonization of enteric

culture samples (crop, cecal contents and cotton swabs) were obtained at day 7, 14, 21, of age of three groups. For three groups the challenge dose was (10^6) cfu *Salmonella typhimurium*. In one ml saline inoculated orally on third day of life.

5- Statistical Analysis

The data were subjected to - CRD test, Chi square was used to determined significance differences ($P < 0.05$) between positive culture samples from crop or ceca with in experiment and T-test for *Salmonella* count (\log_{10})

pathogens (3.6), but at day 14 and 21 there is a significant differences between control and treated groups, although there is no differences between the two treated groups, while control still at high *Salmonella* numbers at day 14 and 21 of age this results may refer to the additional effect of lactose in feed or drinking water, because lactose not hydrolyzed or absorbed intact by the intestinal tract of chickens, because of that (lack of digestion and absorption), lactose pass in to the lower portions of intestine and cecum, the hydrolyzation of lactose dose occur is primarily the results of cecal flora utilization (fermentation) (25), in this study we noticed frequently foamy appearance of cecal contents and ceca is more distension (i.e reduce cecal density due to gas production) in lactose treated groups comparing to control suggested that fermentation of lactose has occurred, these data are analogue to other reports (1,3,13). Microbial fermentation of indigestible carbohydrate results in production of VFA including acetate, propionate and butyrate as well as lactate and lowers PH, these unfavorable conditions have been reported to inhibit *Salmonella* colonization in chickens at 2 weeks of age or older (26, 27). Decrease of *Salmonella* colonization in the chicks provided lactose was associated with significant reduction in the luminal PH and increase acetate, propionate, butyrate and lactate and increased In un dissociated VFA concentrations (2, 10,13), another factor that lactose may induce structural and morphometric changes in the digestive

tract, suggesting the possibility of lactose induced alteration of host susceptibility to *Salmonella* colonization and invasion (11). As results obtained by Hume et al (1992)(28), he indicated that the cecal anaerobes are more likely to convert lactose to VFA and lactic acid than anaerobes in the intestine, although VFA as well as lactic acid are produced throughout the digestive tract. To determine the efficacy of two lactose treatments on *Salmonella* colonization resistance, the protection factor was calculated for each treatment group by dividing mean \log^{10} *Salmonella* of control group at mean \log^{10} *Salmonella* of treatment group as shown in table(2), comparison of the protection factor (pf) of two treatment groups suggests that at termination of the study on day 21, lactose via drinking water was provided a higher level of protection

against colonization comparing to the dietary lactose group, but apparently there is no significant differences between two treatments. Table(3) showed the results of fecal shedding at 7, 14, 21 days of age the results showed although numerically differ, but there are no significant differences between groups, but total positive samples of fecal shedding and total percent are significantly differ between treated groups and control there for it seems to be that the lactose had positive effect on fecal shedding, there for these result showed that decreasing fecal shedding as a whole may had effect to reduce the environmental contamination and poultry houses by *Salmonella* spp., in these study we noticed no significant difference in the body weight as showed in table(4). (i.e. no divers effect on body weight) of chicks received lactose comparing to control.

Table(1) *Salmonella* isolation rate of chicken's crops (positive samples)

Group	Age/days				
	7	14	21	total	%
A	4 ^a /10	2 ^a /10	0 ^a /10	6 ^a /30	20 ^a
B	5 ^a /10	1 ^a /10	1 ^a /10	7 ^a /30	23 ^a
C	9 ^a /10	4 ^a /10	1 ^a /10	14 ^b /30	46 ^b

Results with in one column indicated by different superscripts differ ($P \leq 0.05$)

Table(2) comparison of results obtained from treated and control groups of the *Salmonella* number \log^{10} /gm of cecal contents

Group	Age/days					
	7	pf	14	pf	21	pf
A	5.9 ^a ±.18	1.084	2 ^a ±.5	2.35	1.8 ^a ±.31	1.833
B	5.97 ^a ±.17	1.072	2.6 ^a ±.46	1.807	1.55 ^a ±.3	2.129
C	6.4 ^a ±.09		4.7 ^b ±.52		3.3 ^b ±.36	

Results with in one column indicated by different superscripts differ ($P \leq 0.05$)

Table(3) comparison of results obtained from treated and control groups of fecal shedding

Group	Age/days				
	7	14	21	total	%
A	5 ^a /10	2 ^a /10	2 ^a /10	9 ^a /30	30 ^a
B	6 ^a /10	3 ^a /10	2 ^a /10	11 ^a /30	36 ^a
C	9 ^a /10	7 ^a /10	4 ^a /10	20 ^b /30	66 ^b

Results with in one column indicated by different superscripts differ ($P \leq 0.05$)

Table (4) comparison of results obtained from treated and control groups of body weight

Group	Age/days		
	7	14	21
	Body weight /gm		
A	135 ^a	305 ^a	615 ^a
B	132 ^a	308 ^a	600 ^a
C	140 ^a	310 ^a	610 ^a

Results with in one column indicated no difference between three groups

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دراسة مقارنة لاستخدام سكر اللاكتوز في عليقة الدجاج وماء الشرب في تقليل استيطان جراثيم السالمونيلا لامعاء دجاج اللحم

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الخلاصة

تم في هذا البحث مقارنة استخدام سكر اللاكتوز الممزوج مع العليقة او المقدم عن طريق ماء الشرب لتقليل استيطان جراثيم السالمونيلا للقناة الهضمية (الحوصلة والاعورين) في افراخ اللحم.تم تقسيم الافراخ الى ثلاثة مجاميع كل مجموعة تحتوي على ١٥٠ فرخة،المجموعة الاولى A تم تقديم سكر الاكتوز ممزوجا مع العليقة،المجموعة الثانية B تم تقديم سكر اللاكتوز مع ماء الشرب واعتبرت المجموعة الثالثة C مجموعة سيطرة.تم اعطاء جرعة التحدي عن طريق الفم وكان مقدار الجرعة 10^6 cfu،تم جمع العينات من محتويات الحوصلة والاعورين واخذ المسحات المخرجه بعمر ٢١،١٤،٧ يوم لمعرفة معدل عزل السالمونيلا من الحوصلة وعدد السالمونيلا في محتويات الاعورين ومعدل طرح السالمونيلا.اظهرت النتائج ان هناك انخفاض معنوي في كل من معدل عزل السالمونيلا الكلي من الحوصلة واعداد السالمونيلا من محتويات الاعورين و كذلك انخفاض معنوي لمعدل الطرح الكلي للسالمونيلا من المسحات المخرجة في المجاميع المعاملة بسكر اللاكتوز مقارنة بمجموعة السيطرة،ولا يوجد فارق كبير بين استخدام سكر اللاكتوز في العليقة او ماء الشرب لتقليل استيطان جراثيم السالمونيلا في افراخ اللحم ولم يلاحظ اي تأثير سلبي على الوزن للمجاميع المعاملة بسكر اللاكتوز مقارنة بمجموعة السيطرة (عند وجود فارق معنوي بين المجاميع) خلال فترة التجربة.